distinct type of gene expression library in which a population of polypeptides is generated in such a way that each polypeptide is physically associated with its encoding polynucleotide. The invention relates to a novel method for the generation of display libraries. Gene expression libraries can be generated from a whole host of different nucleotide sources. For example, they can be generated from a population of entirely random nucleotide sequences, from randomly fragmented genomic DNA (genomic libraries) or from the expressed protein complement of a cell or tissue (cDNA libraries). Such libraries, which are well known to those skilled in the art, by definition contain a diverse population of polypeptides of undefined kind, length, structure etc. Display libraries which contain a diverse population of polypeptides of undefined kind, length, structure etc., are also well known to those skilled in the art.

The defining benefit of the display library concept is that the display mechanism itself provides a solution to the formidable task of screening a library containing a diverse population of expressed polypeptides to identify those library members that have a desired property (for example, the ability to bind to a target molecule). Those skilled in the art are well aware of the possibilities

available for screening for the property under investigation. With traditional, non-display gene expression libraries, those members of the library that possess the required property have to be identified by screening individual library clones that have been arrayed on a solid support. This need to array each member of the library imposes practical limits on the size and complexity of libraries that can be screened due to the laboratory space that would be required to array ever more clones and the concomitant effort that would be required to screen them. Display libraries overcome this constraint since an entire display library is screened as a single pool of polypeptide/polynucleotide complexes, for example:

- the library is incubated with a target molecule,
- non-binding complexes are removed by washing,
- this leaves a reduced population of library members that bind to the target molecule which can be released from the target using suitable elution techniques.

The most important feature of any display library is the physical association of each polypeptide to its own encoding polynucleotide. This physical link enables the recovery of the eluted polynucleotides that encode the

binding polypeptides as these can be readily amplified (e.g., by cloning or by PCR) for further manipulation, analysis or storage.

A major benefit of using display libraries is that very much larger libraries can be generated and screened than would be possible using a non-display library approach. This results in an increased probability that a polypeptide with the desired properties will be identified.

Applicants' invention relates to a new method of physically linking each polypeptide within a display library with its own encoding polynucleotide. Applicants have recognized that it is possible to construct a display library using a protein which, during the combined processes of transcription and translation, binds *spontaneously* and *faithfully* through covalent bonding to its *own* encoding DNA (a property called *cis*-acting DNA binding).

Applicants' invention uses this property of *cis*-acting DNA binding to make a display library. To achieve this, a population of DNA molecules is constructed in which each DNA molecule includes two in-frame polypeptide coding sequences. The first sequence contains the coding sequence for the *cis*-acting DNA binding protein and the second

sequence encodes one of a diverse population of polypeptides for display.

The method of the present invention can utilize any *cis*-acting DNA binding protein. The invention does not specifically relate to the identification of novel proteins that share this ability to bind DNA *in cis* but to the use of a *cis*-acting DNA binding protein to provide the physical link between an amino acid sequence and the DNA sequence that has encoded that amino acid sequence.

The invention relates to the recognition that a class of proteins exists that is *cis*-acting DNA binding proteins that can be applied to the generation of a display library. One skilled in the art seeking to work the invention across the breadth of the claim is provided with clear instructions concerning the properties of the *cis*-acting DNA binding protein. A number of examples of suitable proteins are provided in the specification, see for example the disclosure beginning on page 7 of the description.

As described above, the proteins expressed in the present method have two components, a *cis*-acting DNA binding portion and a variable display portion. A diverse population of amino acid coding sequences for the display portion are provided within the library. By its very nature, a library relates to a variety of different

proteins and not to a single protein. Thus, where on page 3 of the Office Action, the Examiner states:

> "Assuming for the sake of arguments, that one is able to produce a library of not a single protein but a diverse population of proteins"

the Examiner appears to have misinterpreted the nature of Applicants' invention. In Applicants' invention, each protein in the library has the ability to bind to its own encoding DNA via a 'binding domain' which spontaneously and faithfully forms a covalent bond with its own encoding DNA during transcription and translation. Each of the proteins in the library also has a second domain, the 'display moiety'. It is the display moieties that are derived from a diverse population of encoding DNA sequences that result in the display of a diverse population of peptides. It is not necessary to find a method of screening that would enable all of the peptides or proteins in the library to be isolated and tested. The screening method is used to identify the single or few peptides or proteins in the library that have the desired property. Screening methods are readily available to enable the identification of polypeptides with desired properties from a diverse

population of peptides or proteins. Thus, for example, if the 'desired property' is the ability of the display moiety to bind to a particular receptor, it is probable that the majority of proteins in the library would not possess that property. However, in generating libraries displayed moieties that do possess the desired property can be identified. The polypeptides containing display moieties with the desired property are separated from those that do not possess the desired property by, for example, affinity selection using an immobilised preparation of the target receptor. Many methods for immobilising target molecules and for affinity selection exist and are well known to those skilled in the art.

During the selection process, the DNA molecules that encode proteins that contain display moieties with the desired property can be separated from those DNA molecules that do not because all of the proteins in the sample are covalently bound to their own encoding DNA via the 'binding portion'. The DNA molecules that encode proteins that contain the display moieties with the desired property can be recovered for amplification, storage, or further manipulation and analysis.

The claims have been revised to define the invention with additional clarity. The claims as presented are fully

supported by an enabling disclosure. Thus, the rejections under 35 USC 112 are believed moot. More specifically, claim 1 has been revised as new claim 19 to clarify the components of the claim to make it clear that it is the display moiety itself which has one site of attachment for the binding moiety. Claim 1 has also been revised to incorporate the feature that the amino acid sequence of the binding moiety is a *cis*-acting protein. Claim 14 (revised as new claim 31) essentially relates to the use of the library as defined in claim 11 (new claim 28). The library will be composed of a diverse population of library members. However, the screening method is designed to isolate only those proteins or peptides from that diverse population that have the desired characteristics. It is not expected that any screening method should be required which enables selection and isolation of all of the library members. Such a screening method is common practice in the use of display libraries.

Novelty and Non Obviousness

Applicants submit that the claims are novel and not obvious over Mattheakis *et al*.

Mattheakis *et al* describes the production of an expression library using polysome display technology (or sometimes referred to as ribosome display technology).

Polysome display works by establishing a physical link between a nascent polypeptide and its encoding polynucleotide, that is mRNA, using ribosomes. *In vitro* translation conditions are adopted that result in the stalling of translating ribosomes so that they simultaneously remain attached to the encoding polynucleotide and to the encoded nascent polypeptide. The polysomes so formed are then isolated in a stable form for subsequent screening.

Thus, in the polysome display technique described in Mattheakis *et al*, a protein is bound to its encoding mRNA via the polysome. There is no covalent protein to DNA bonding, and the protein is not bound to its own encoding DNA.

Mattheakis has modified this general method to include a tether segment. The tether segment is used so that the polysomes can be removed prior to screening. The disclosure beginning in column 31 of the Detailed Description under the heading "Tether-binding antibody linked to polynucleotide" describes the tethers that may be used in accordance with the Mattheakis invention. Firstly,

in one embodiment, an antibody is bound to the mRNA polynucleotide sequence. This antibody binding is through avidin-biotin bridge or via cross linkers. The polynucleotide encodes an epitope of the antibody such that the antibody will bind to its epitope upon expression of the polynucleotide. In the alternative, binding is carried out using biotin/streptavadin system, including an enzyme to biotinylate the nascent peptide which is then able to bind to streptavidin bound to the mRNA polynucleotide. Finally, RNA binding proteins are disclosed.

All of these methods initially require polysome production and a subsequent stabilisation of the link between the nascent peptide and encoding polynucleotide through interaction between the tether segment and its target ligand associated with the encoding polypeptide. There is no disclosure of any DNA binding protein which binds to its own encoding DNA through covalent bonds.

The present invention provides a much simpler system than that described in Mattheakis *et al* which does not require a polysome display step. It is not necessary to establish translation conditions which would permit the tether segment of the nascent polypeptide to bind to the polynucleotide via an intermediate before significant association and diffusion of the nascent peptide from the

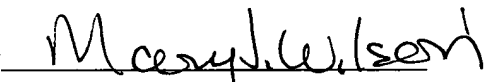
translation complex occurs. In accordance with the present application, the link between nascent polypeptide and its encoding polynucleotide forms spontaneously during the couple transcription and translation of the *cis*-acting DNA binding protein. Mattheakis *et al* do not mention the use of a covalent DNA-binding protein to link the nascent polypeptide and the encoding polynucleotide. There is no suggestion in Mattheakis that a simpler solution could be provided using the *cis*-acting proteins of the present invention.

Reconsideration is hereby requested.

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

NIXON & VANDERHYE, P.C.

By 
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